PATENT
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Client Ref. No.: A-68983-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation Number: 2359
David A. Horwitz	Examiner: Juedes, Amy E.
Application No.: 10/772,768	Technology Center/Art Unit: 1644
Filed: February 4, 2004	DECLARATION OF DAVID HORWITZ UNDER 37 C.F.R. §1.132
For: *Method to Prevent Graft Rejection Using TGF-Beta to Induce T Suppressor Cells*	
Customer No.: 67374	

Commissioner for Patents
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I, David A. Horwitz, M.D., having a residence at 566 Latima Road, Santa Monica, CA, and being a U.S. citizen, do hereby declare as follows:

1. I am the inventor on the above-identified patent application and am familiar with its contents. I have also reviewed the pending claims in this application.

2. I have read the Examiner's comments in the Office Action mailed on October 10, 2006. It is my understanding that the Examiner has rejected claims 1-6 primarily because the suppressor T cells recited in the pending claims are allegedly anticipated under 35 U.S.C. §102(e) by McIntosh et al. U.S. Patent No. 6,685,936 (hereinafter "the '936 patent") and under 35 U.S.C. §102(b) by Hall et al. J Exp Med 1990 171:141-157 (hereinafter "Hall").

3. The Examiner cites the '936 patent as teaching suppressor T cells capable of treating (i.e. decreasing) transplant rejection and that such cells may be CD8+. Hall is cited

by the Examiner for teaching CD4+ suppressor T cells capable of inhibiting restoration of transplant rejection (i.e., decreasing transplant rejection).

4. I am submitting this declaration to demonstrate that the presently claimed suppressor T cells differ from the suppressor T cells disclosed in either Hall or the '936 patent. As I explained in a declaration dated August 19, 2006, the presently claimed CD4+ suppressor T cells have suppressive activity independent of CD8+ cells, while the cells of both Hall and the '936 patent require the presence of CD8+ cells for CD4+ cells to develop suppressive activity. It is my understanding that the Examiner believes that Hall teaches that CD8+ cells do not mediate suppression (*Office Action, p. 4, second paragraph*). This assertion by the Examiner is incorrect. In Table V, Hall clearly shows that radioresistant CD8+ cells are required for the CD4+ suppressor cells to function, and on the next page, Hall explicitly states: "Taken together, these results show the rejection response in irradiated rats is inhibited by an MRC Ox8+ cell that was radioresistant but not thymus derived. This cell was critical for the transfer of suppression by the W3/25+ cells from CSA-treated hosts." *emphasis added, Hall, p.148, second full paragraph.* The W3/25+ cells of Hall are CD4+ cells, and the MRC Ox8+ cells are CD8+ cells. Therefore, Hall clearly concludes that the CD4+ cells in its system require the presence of CD8+ cells to mediate suppressive activity. This is not true for the claimed CD4+ suppressor cells.

5. In the methods described in the present application, either CD4+ or CD8+ cells can be induced to become the claimed suppressor cells, but neither requires the presence of the other to develop suppressive activity.

6. The CD8+ cells that can be induced to become suppressor T cells using the methods of the present application are also not the same suppressor T cells as disclosed in either Hall or the '936 patent. CD8+ suppressor cells have been shown to be CD28 negative (see Exhibits 2 and 3 of the accompanying response to office action and Exhibit B of this declaration). In the present invention, naïve CD8+ cells are used as our starting population to make suppressor T cells, and these T cells are CD28 positive (see Exhibit B).

7. Foxp3 has been identified as the transcription factor that is critical for the differentiation of T cells, and there is now universal agreement in the literature that TGF-β induces Foxp3 expression, which is supported by the data in the enclosed Exhibit A. For the experiments of Exhibit A, the starting population was naive human CD8+CD54RO- cells, a subset that characteristically expresses CD28. These CD8+ cells were stimulated with beads coated with anti-CD3 and anti-CD28 plus IL-2 in the presence or absence of TGF-β. On day 6, the cells were assayed for Foxp3 (abscissa) and CD25 expression (ordinate) by flow cytometry (right panel). As Exhibit A shows, the methods and compositions of this invention induce CD8+ suppressor cells which are Foxp3 positive. Such CD8+CD28+Foxp3+ suppressor cells are rare in the blood and lymphoid organs of mice and humans, as discussed in the reference enclosed herewith as Exhibit B, which shows that naive CD8+ cells lose expression of the CD28 marker when they become cytolytic effector cells. Thus, we can clearly differentiate the suppressor cells described in both references cited by the Examiner from those we generate *ex vivo*.

8. I declare further that all statements made herein of my own knoweldge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 10, 2007 By: _____
David A. Horwitz, M.D.

Enclosures:
Exhibit A: data on CD8+ suppressor cells expression profile
Exhibit B: Hamann et al., *J. Exp. Med.*, (1997), 186(9): 1407-18.